

PATENT COOPERATION TREATY

PCT

REC'D 23 NOV 2005

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY PCT

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P34235A/CMUMCM	FOR FURTHER ACTION <div style="text-align: right;">See Form PCT/PEA/416</div>	
International application No. PCT/GB2004/003391	International filing date (day/month/year) 05.08.2004	Priority date (day/month/year) 05.08.2003
International Patent Classification (IPC) or national classification and IPC C07K1/107, C07K19/00		
Applicant CSS-ALBACHEM LIMITED et al.		
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 5 sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau) a total of 9 sheets, as follows: <input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). <input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).		
4. This report contains indications relating to the following items: <div style="margin-left: 20px;"> <input checked="" type="checkbox"/> Box No. I Basis of the opinion <input checked="" type="checkbox"/> Box No. II Priority <input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability <input type="checkbox"/> Box No. IV Lack of unity of invention <input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement <input type="checkbox"/> Box No. VI Certain documents cited <input type="checkbox"/> Box No. VII Certain defects in the international application <input type="checkbox"/> Box No. VIII Certain observations on the international application </div>		
Date of submission of the demand 03.06.2005	Date of completion of this report 22.11.2005	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized Officer Mundel, C Telephone No. +49 89 2399-7314	



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003391

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-57 as originally filed

Sequence listings part of the description, Pages

1-5 received on 24.11.2004 with letter of 23.11.2004

Claims, Numbers

1-27 received on 09.06.2005

Drawings, Sheets

1/15-15/15 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003391

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-27
	No: Claims	
Inventive step (IS)	Yes: Claims	1-27
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-27
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. The present application refers to methods for producing oligopeptide products wherein a first oligopeptide product and a second oligopeptide / label molecule are linked via a linking moiety having formula I, formula II or formula III. The application also refers to labelled oligopeptides produced by such methods.
2. Reference is made to the following documents :
 - D1: PERLER F.B. ET AL.: "The mechanism of protein splicing: variations on a theme" PEPTIDES 2002, 2002, pages 254-255, NAPOLI, ITALY
 - D2: CHONG S ET AL: "Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 192, no. 2, 1997, pages 271-281.
 - D3: COTTON GRAHAM J ET AL: "Peptide ligation and its application to protein engineering" CHEMISTRY AND BIOLOGY (LONDON), vol. 6, no. 9, September 1999 (1999-09), pages R247-R256.
 - D4: WO 00/18881 A (XU MING QUN ; NEW ENGLAND BIOLABS INC (US); EVANS THOMAS C (US)) 6 April 2000 (2000-04-06)
 - D5: GEOGHEGAN K F: "Site-directed conjugation of nonpeptide groups to peptides and proteins via periodate oxidation of a 2-amino alcohol. Application to modification at N-terminal serine" BIOCONJUGATE CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 3, no. 2, 1992, pages 138-146.
3. **Novelty; article 33(2) PCT.**

The subject-matter of claims 1-27 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 1-27 have to be considered as novel in the sense of Article 33(2) PCT.

4. Inventive step; article 33(3) PCT.

The documents D1 to D4 disclose the use of peptides linked to a modified intein for the generation of peptides having an activated C-terminal α thioester. This technique has been used for Expressed Protein Ligation or Intein-mediated Protein Ligation where the second peptide possesses a N-terminal cysteine residue which reacts with the thioester to form a peptide bond.

Even of the documents D1 and D4 refer to a general nucleophilic attack, all the examples disclosed in said documents involve the attack of the C-terminal thioester of a recombinant peptide by a peptide having a N-terminal cysteine.

None of the documents cited in the International Search Report suggest the methods and products of the present application

Therefore, the subject-matter of claims 1-27 has to be considered as inventive in the sense of article 33(3) PCT.

1 **Claims**

2

3 1. A method of producing an oligopeptide product,
4 the method comprising the steps:

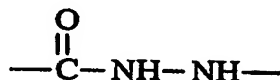
5 a) providing a first oligopeptide, the first
6 oligopeptide having a reactive moiety,

7 b) providing a second oligopeptide, the second
8 oligopeptide having a activated ester moiety

9 c) allowing the reactive moiety of the first
10 oligopeptide to react with the activated ester
11 moiety of the second oligopeptide to form an
12 oligopeptide product, in which the first and second
13 oligopeptides are linked via a linking moiety having
14 Formula I, Formula II or Formula III.

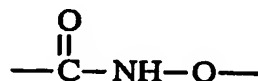
15

16 Formula I



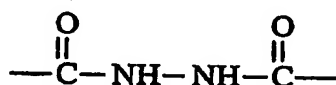
17

18 Formula II



19

20 Formula III



21

22

23

24 2. The method according to claim 1 wherein the
25 terminal activated ester moiety is a thioester
26 wherein the peptide is the acyl substituent of

- 1 the thioester.
- 2
- 3 3. The method according to claim 2, wherein said
- 4 second polypeptide is generated by thiol reagent
- 5 dependent cleavage of a precursor molecule, said
- 6 precursor molecule comprising a second oligopeptide
- 7 fused N-terminally to an intein domain.
- 8
- 9 4. A method of producing an oligopeptide product,
- 10 the method comprising the steps:
- 11 a) providing a first oligopeptide, the first
- 12 oligopeptide having a reactive moiety,
- 13 b)i) providing a precursor oligopeptide molecule,
- 14 the precursor oligopeptide molecule comprising a
- 15 second oligopeptide fused N-terminally to an intein
- 16 domain
- 17 ii) allowing thiol reagent dependent cleavage of the
- 18 precursor molecule to generate a second oligopeptide
- 19 molecule, said second oligopeptide molecule having a
- 20 thioester moiety at its C-terminus,
- 21 c) allowing the reactive moiety of the first
- 22 oligopeptide to react with the second oligopeptide
- 23 molecule to form an oligopeptide product, in which
- 24 the first and second oligopeptides are linked via a
- 25 linking moiety having Formula I, II or III.
- 26
- 27 5. The method according to any one of the preceding
- 28 claims wherein the reactive moiety is a hydrazine
- 29 moiety, a hydrazide moiety or an aminooxy moiety.
- 30
- 31 6. The method according to claim 5, wherein the
- 32 reactive moiety is an aminooxy moiety and the

- 1 activated ester moiety is a thioester.
2
- 3 7. The method according to claim 5, wherein said
4 first oligopeptide is produced by reaction of
5 hydrazine with a precursor molecule, said
6 precursor molecule comprising a precursor
7 oligopeptide fused N-terminally to an intein
8 domain via a thioester moiety.
9
- 10 8. A method of producing an oligopeptide product,
11 said method comprising the steps:
12 a) providing a first oligopeptide, the first
13 oligopeptide having a reactive moiety, wherein
14 the reactive moiety is a hydrazine moiety, a
15 hydrazide moiety or an amino-oxy moiety;
16 b) providing a precursor oligopeptide molecule,
17 the precursor oligopeptide molecule comprising a
18 second oligopeptide fused N-terminally to an
19 intein domain;
20 c) allowing the reactive moiety of the first
21 oligopeptide to react with the precursor
22 oligopeptide molecule to form an oligopeptide
23 product, in which the first and second
24 oligopeptides are linked via a linking moiety
25 having Formula I, Formula II or Formula III.
26
- 27 9. The method according to any one of the preceding
28 claims, wherein the first oligopeptide or the
29 second oligopeptide is a recombinant oligopeptide
30 and the other of the the first oligopeptide and
31 the second oligopeptide is a synthetic
32 polypeptide.

- 1
- 2 10. The method according to any one of claims 1 to
- 3 8, wherein the first oligopeptide and the second
- 4 oligopeptide are recombinant oligopeptides.
- 5
- 6 11. The method according to any one of claims 1 to
- 7 8, wherein the first oligopeptide and the second
- 8 oligopeptide are synthetic oligopeptides.
- 9
- 10 12. A method of generating a protein hydrazide,
- 11 said method comprising the steps:
- 12 (a) providing a protein molecule comprising an
- 13 oligopeptide fused N-terminal to an intein
- 14 domain,
- 15 (b) reacting said protein molecule with
- 16 hydrazine, such that the intein domain is cleaved
- 17 from the oligopeptide to generate a protein
- 18 hydrazide.
- 19
- 20 13. The method according to any one of the claims 1
- 21 to 11 wherein step (c) of the method is performed
- 22 at a pH in the range pH 6.5 to 7.5.
- 23
- 24 14. A method of producing an oligopeptide product,
- 25 the method comprising the steps:
- 26 a) providing a first oligopeptide, the first
- 27 oligopeptide having an aldehyde or ketone moiety,
- 28 b) providing a precursor oligopeptide molecule,
- 29 the precursor oligopeptide molecule comprising a
- 30 second oligopeptide fused N-terminally to an
- 31 intein domain,
- 32 c) reacting said precursor oligopeptide molecule

- 1 with hydrazine to generate an oligopeptide
- 2 molecule comprising an intermediate oligopeptide,
- 3 said intermediate oligopeptide having a terminal
- 4 hydrazide moiety,
- 5 d) allowing the aldehyde or ketone moiety of the
- 6 first oligopeptide to react with the hydrazide
- 7 moiety of the intermediate oligopeptide molecule
- 8 to form an oligopeptide product, in which first
- 9 oligopeptide and the second oligopeptide are
- 10 linked via a hydrazone linking moiety.
- 11
- 12 15. An oligopeptide product produced by the method
- 13 of any one of the preceding claims, in which the
- 14 first and second oligopeptides are linked via a
- 15 linking moiety having Formula II or Formula III.
- 16
- 17 16. A method of labelling an oligopeptide, the
- 18 method comprising the steps:
- 19 a) providing a label molecule, the label molecule
- 20 having a reactive moiety,
- 21 b) providing the oligopeptide, the oligopeptide
- 22 having a activated ester moiety
- 23 c) allowing the reactive moiety of the label
- 24 molecule to react with the activated ester moiety
- 25 of the oligopeptide to form the labelled
- 26 oligopeptide, in which the label molecule and the
- 27 oligopeptide are linked via a linking moiety
- 28 having Formula I, Formula II or Formula III.
- 29
- 30 17. The method according to claim 16, wherein in
- 31 step (c), where said label molecule and the
- 32 oligopeptide are linked via a linking moiety

1 having Formula II and where said activated ester
2 moiety of step (b) is not a thioester, said
3 activated ester is a terminal activated ester
4 moiety.

5

6 18. A method of labelling an oligopeptide, the
7 method comprising the steps:
8 a) providing a label molecule, the label molecule
9 having an activated ester moiety of which the
10 label is the acyl substituent,
11 b) providing the oligopeptide, the oligopeptide
12 having a reactive moiety
13 c) allowing the activated ester moiety of the
14 label molecule to react with the reactive moiety
15 of the oligopeptide to form the labelled
16 oligopeptide, in which the label molecule and the
17 oligopeptide are linked via a linking moiety
18 having Formula I, Formula II or Formula III,
19 wherein, in step (c), where said label molecule
20 and the oligopeptide are linked via a linking
21 moiety having Formula II and where said activated
22 ester moiety of step (b) is not a thioester, said
23 activated ester is a terminal activated ester
24 moiety.

25

26 19. The method according to claim 18 wherein said
27 oligopeptide is produced by reaction of hydrazine
28 with a precursor molecule, said precursor
29 molecule comprising a precursor oligopeptide
30 fused N-terminally to an intein domain via a
31 thioester moiety.

32

- 1 20. A method of labelling an oligopeptide, the
2 method comprising the steps:
3 a) providing a label, the label having a reactive
4 moiety,
5 b) (i) providing a precursor oligopeptide
6 molecule, the precursor oligopeptide molecule
7 comprising an oligopeptide fused N-terminally to
8 an intein domain
9 (ii) allowing thiol reagent dependent cleavage of
10 the precursor molecule to generate the
11 oligopeptide molecule, said oligopeptide molecule
12 having a thioester moiety at its C-terminus,
13 c) allowing the reactive moiety of the label to
14 react with the oligopeptide molecule to form a
15 labelled oligopeptide, in which the label and
16 oligopeptide are linked via a linking moiety
17 having Formula I, II or III.
18
- 19 21. The method according to any one of claims 16 to
20 18, wherein the reactive moiety is an aminooxy
21 moiety and the activated ester moiety is a
22 thioester.
23
- 24 22. The method according to claim 20, wherein the
25 reactive moiety is an aminooxy moiety.
26
- 27 23. A method of labelling an oligopeptide, the
28 method comprising the steps:
29 a) providing a label molecule, the label molecule
30 having a reactive moiety,
31 b) providing a precursor oligopeptide molecule,
32 the precursor oligopeptide molecule comprising an

1 oligopeptide fused N-terminally to an intein
2 domain,
3 c) allowing the reactive moiety of the label
4 molecule to react with the precursor oligopeptide
5 molecule to form a labelled oligopeptide product,
6 in which the label molecule and the oligopeptide
7 are linked via a linking moiety having Formula I,
8 Formula II or Formula III as defined above.

9
10 24. The method according to any one of claims 16 to
11 23 wherein step (c) of the method is performed at
12 a pH in the range pH 6.5 to pH 7.5.

13
14 25. A method of labelling an oligopeptide, the
15 method comprising the steps:
16 a) providing a label molecule, the label molecule
17 having a aldehyde or ketone moiety,
18 b) providing a precursor oligopeptide molecule,
19 the precursor oligopeptide molecule comprising a
20 first oligopeptide fused N-terminally to an
21 intein domain,
22 c) reacting said precursor oligopeptide molecule
23 with hydrazine to generate an oligopeptide
24 molecule comprising an intermediate oligopeptide,
25 said intermediate oligopeptide having a terminal
26 hydrazide moiety,
27 d) allowing the aldehyde or ketone moiety of the
28 label molecule to react with the hydrazide moiety
29 of the intermediate oligopeptide molecule to form
30 a labelled oligopeptide product, in which the
31 label molecule and oligopeptide are linked via a

1 hydrazone linking moiety.

2

3 26. The method according to claim 14 or claim 25,
4 wherein the aldehyde or ketone moiety is an α -
5 diketone or an α -keto-aldehyde group.

6

7 27. A labelled oligopeptide produced by the method
8 of any one of claims 16 to 26, in which the first
9 and second oligopeptides are linked via a linking
10 moiety having Formula II or Formula III.

11

12